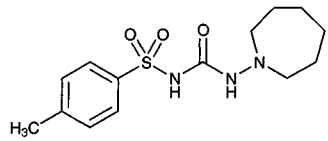
Tolazamide



Molecular formula: $C_{14}H_{21}N_3O_3S$
Molecular weight: 311.41
CAS Registry No.: 1156-19-0
Merck Index: 9644
Lednicer No.: 1 241

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 500 μL 5-(p-methylphenyl)-5-phenylhydantoin in chloroform + 500 μL pH 4.5 sodium acetate buffer + 5 mL dichloromethane, shake horizontally for 5 min, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 80 μL MeOH, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb C-18
Mobile phase: MeOH:pH 5.6 acetate buffer 52.3:47.7
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5
Internal standard: 5-(p-methylphenyl)-5-phenylhydantoin (7.5)
Limit of quantitation: 1000 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

Welling,P.G.; Patel,R.B.; Patel,U.R.; Gillespie,W.R.; Craig,W.A.; Albert,K.S. Bioavailability of tolazamide from tablets: comparison of in vitro and in vivo results, *J.Pharm.Sci.*, **1982**, *71*, 1259–1263.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 2 mL water + 200 μL 1 (?) M HCl + 200 μL 2.5 μg/mL glibornuride in MeOH + 7 mL diethyl ether, mix, centrifuge at 2000 rpm for 5 min. Remove 6.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 2 mg/mL dinitrofluorobenzene in butyl acetate, heat at 120° for 1 h, cool, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 120 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS 2
Mobile phase: MeCN:0.4% aqueous phosphoric acid 75:25
Column temperature: 40
Flow rate: 1.2
Injection volume: 120
Detector: UV 360

CHROMATOGRAM

Retention time: 7.3
Internal standard: glibornuride (5.8)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide, glipizide, glyburide (glibenclamide), tolbutamide

KEY WORDS
serum; derivatization

REFERENCE
Starkey,B.J.; Mould,G.P.; Teale,J.D. The determination of sulphonylurea drugs by HPLC and its clinical application, *J.Liq.Chromatogr.*, **1989**, *12*, 1889–1896.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 2 M HCl + 2 mL diethyl ether, vortex for 30 s, centrifuge at 1500 g for 5 min, freeze in dry ice for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject a 25-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18 (Alltech)
Mobile phase: MeCN:10 mM orthophosphoric acid 50:50
Flow rate: 1
Injection volume: 25-50
Detector: UV 230

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Extracted: chlorpropamide, gliclazide, glyburide (glibenclamide), glipizide, tolbutamide
Simultaneous: sulfamethoxazole, N-acetylsulfamethoxazole
Noninterfering: trimethoprim

KEY WORDS
plasma

REFERENCE
Shenfield,G.M.; Boutagy,J.S.; Webb,C. A screening test for detecting sulfonylureas in plasma, *Ther.Drug Monit.*, **1990**, *12*, 393–397.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

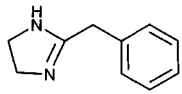
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, dil-

tiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Tolazoline



Molecular formula: $C_{10}H_{12}N_2$
Molecular weight: 160.22
CAS Registry No.: 59-98-3, 59-97-2 (HCl)
Merck Index: 9645

SAMPLE

Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 2.5 μg/mL clonidine in 10 mM HCl + 50 μL 3 M NaOH + 1.5 mL n-butyl chloride:isopropanol 95:5, vortex 60 s, centrifuge at 2500 rpm (20 cm) for 2 min. Freeze in dry ice/isopropanol for 20 s and remove organic phase. Add 100 μL 50 mM phosphoric acid to organic phase, vortex 60 s, centrifuge at 2500 rpm (20 cm) for 2 min, remove organic layer, inject 25 μL aliquots of aqueous layer.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-PCN
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KOH/H_3PO_4, pH 3.0.)
Column temperature: 35
Flow rate: 2
Injection volume: 25
Detector: UV 214

CHROMATOGRAM
Retention time: 3.7
Internal standard: clonidine
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: amikacin, ampicillin, dopamine, furosemide, gentamicin, heparin, morphine, pancuronium

KEY WORDS
serum

REFERENCE
Todesco,L.M.; Thoma,J.J.; Barth,R.D.; Myers,N.J.; White,R.; Ward,R.M. Quantitative determination of tolazoline in human serum by high performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 78–84.

SAMPLE
Matrix: blood, urine
Sample preparation: 50-100 μL Serum or urine + 30 μL 1 (serum) or 100 (urine) μg/mL naphazoline + 250 μL buffer + 5 mL dichloromethane, shake mechanically for 20 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 (serum) or 500 (urine) μL 20 mM pH 3.75 KH_2PO_4, vortex for 15 s, inject a 10 (urine) or 90 (serum) μL aliquot. (Buffer was 100 mM potassium hydrogen carbonate and 100 mM potassium carbonate, pH 10.0.)

HPLC VARIABLES
Guard column: 50 × 2 Co:Pell ODS
Column: Resolve C18 (Waters)
Mobile phase: MeCN:buffer 40:60 (Buffer was 20 mM KH_2PO_4 adjusted to pH 3.75 with 85% phosphoric acid.)
Flow rate: 1.2
Injection volume: 10-90
Detector: UV 210

CHROMATOGRAM
Retention time: 7
Internal standard: naphazoline (10)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, carbamazepine, chloramphenicol, desipramine, digoxin, disopyramide, dopamine, ethosuximide, gentamicin, imipramine, lidocaine, methotrexate, phenobarbital, phenytoin, primidone, procainamide, propranolol, quinidine, salicylic acid, theophylline, valproic acid

KEY WORDS
serum

REFERENCE
Cwik,M.J.; Chiu,G.P.; Fischer,J.H.; Chow-Tung,E.; Currie,B.L. Quantitative determination of tolazoline in serum and urine, *J.Chromatogr.*, **1985**, *338*, 123–130.

SAMPLE
Matrix: formulations
Sample preparation: Mix 5 mL nasal solution and 10 mL 500 μg/mL tolazoline hydrochloride in MeOH:water 40:60, make up to 50 mL with MeOH:water 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 RSIL C18 (RSL, Eke, Belgium)
Mobile phase: MeOH:water 40:60 containing 20 mM sodium 1-octanesulfonate and 10 mM N,N-dimethyloctylamine, pH adjusted to 3.0 with orthophosphoric acid

Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 2.5
Internal standard: tolazoline

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline, coumazoline, lidocaine, naphazoline, oxymetazoline, prednisolone, sulfadimidine, sulfanilamide, sulfathiazole, tenaphtoxaline, tetrahydrozoline, tramazoline, xylometazoline

KEY WORDS
nasal solutions; stability-indicating; tolazoline is IS

REFERENCE
De Schutter,J.A.; Van den Bossche,W.; De Moerloose,P. Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography, *J.Chromatogr.*, **1987**, *391*, 303–308.

SAMPLE
Matrix: solutions

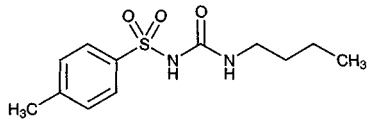
HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic

acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Tolbutamide



Molecular formula: $C_{12}H_{18}N_2O_3S$
Molecular weight: 270.35
CAS Registry No.: 64-77-7, 473-41-6 (Na salt)
Merck Index: 9646
Lednicer No.: 1 136

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 60 ng chlorpropamide + 500 µL 1 M HCl + 8 mL chloroform, shake on a reciprocal shaker, shake for 10 min in a reciprocal shaker, centrifuge at 2000 g for 15 min. Remove 7 mL of the lower organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 µL 3 mg/mL dinitrofluorobenzene in n-butyl acetate, heat at 120° for 30 min, evaporate to dryness under a stream of nitrogen at 60°, dissolve the residue in 100 µL mobile phase, inject a 30-70 µL aliquot. (Recrystallize dinitrofluorobenzene from diethyl ether. Prepare solutions weekly, store at 4° in the dark.)

HPLC VARIABLES

Column: 125 × 4.6 5 µm C8 (Perkin-Elmer)
Mobile phase: MeCN:water 50:50 containing 0.15% phosphoric acid
Flow rate: 1.5
Injection volume: 30-70
Detector: UV 350

CHROMATOGRAM

Retention time: 4.5
Internal standard: chlorpropamide (6.2)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: glyburide
Noninterfering: acetaminophen, aspirin, diazepam, chlordiazepoxide, quinidine, phenytoin, theophylline, phenobarbital

KEY WORDS

plasma; derivatization

REFERENCE
Zecca,L.; Trivulzio,S.; Pinelli,A.; Colombo,R.; Tofanetti,O. Determination of glibenclamide, chlorpropamide and tolbutamide in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1985**, *339*, 203–209.

SAMPLE
Matrix: blood
Sample preparation: 1 mL PLasma or urine + 10 μL MeOH + 10 μL concentrated HCl + 8 mL toluene, extract for 15 min, centrifuge at 1500 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm C18 (Merck)
Mobile phase: MeOH:50 mM pH 5.0 phosphate buffer 50:50
Flow rate: 1
Injection volume: 5
Detector: UV 230

CHROMATOGRAM
Retention time: 6
Internal standard: tolbutamide

OTHER SUBSTANCES
Extracted: nimesulide
Simultaneous: acetaminophen, aspirin, doxepin, glibenclamide, salicylic acid, theophylline
Noninterfering: digoxin, flurazepam, tiadenol
Interfering: bezafibrate

KEY WORDS
plasma; tolbutamide is IS

REFERENCE
Castoldi,D.; Monzani,V.; Tofanetti,O. Simultaneous determination of nimesulide and hydroxynimesulide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *425*, 413–418.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 2 mL 2 M HCl + 1 mL 2.5 μg/mL chlorpropamide in water + 5 mL diethyl ether, extract, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL DMF:water 10:90, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 μm μBondapak C16
Column: 250 × 2.5 5 μm Apex ODS (Rayonic Scientifique)
Mobile phase: MeCN:buffer 22.5:77.5 containing 3 mL/L Pic A (tetrabutylammonium phosphate) (Buffer was 392 mL 67 mM KH_2PO_4 and 608 mL 67 mM Na_2HPO_4, pH 7.0.)
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 14.8
Internal standard: chlorpropamide (10.0)
Limit of detection: 100-200 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; serum; pharmacokinetics

REFERENCE

St-Hilaire,S.; Belanger,P.M. Simultaneous determinations of tolbutamide and its hydroxy and carboxy metabolites in serum and urine: application to pharmacokinetic studies of tolbutamide in the rat, *J.Pharm.Sci.*, **1989**, *78*, 863–866.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Serum + 2 mL water + 200 μL 1 (?) M HCl + 200 μL 2.5 μg/mL glibornuride in MeOH + 7 mL diethyl ether, mix, centrifuge at 2000 rpm for 5 min. Remove 6.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 2 mg/mL dinitrofluorobenzene in butyl acetate, heat at 120° for 1 h, cool, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 120 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS 2
Mobile phase: MeCN:0.4% aqueous phosphoric acid 75:25
Column temperature: 40
Flow rate: 1.2
Injection volume: 120
Detector: UV 360

CHROMATOGRAM

Retention time: 4.9
Internal standard: glibornuride (5.8)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide, glipizide, glyburide (glibenclamide), tolazamide

KEY WORDS

serum; derivatization

REFERENCE

Starkey,B.J.; Mould,G.P.; Teale,J.D. The determination of sulphonylurea drugs by HPLC and its clinical application, *J.Liq.Chromatogr.*, **1989**, *12*, 1889–1896.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 200 μL 1 M HCl + 10 μg glyburide + 5 mL toluene, shake gently for 15 min, centrifuge at 1500 g for 3 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 25 μL 6 mg/mL dinitrofluorobenzene in n-butyl acetate (prepare fresh each week, store at 4° in the dark), heat at 120° for 30 min, evaporate to dryness, reconstitute with 50 μL mobile phase, inject a 25-50 μL aliquot. Alternatively, filter (Amicon YMT membrane, 30000 MW cutoff) 200 μL 100 mM NaOH while centrifuging at 4°, rinse filter with 500 μL water, filter 1 mL serum in the same unit while centrifuging at 4° at 2500 g for 1.5 h. Remove a 700 μL aliquot of the ultrafiltrate, add 200 μL 1 M HCl, add 10 μg glyburide, add 5 mL toluene, shake gently for 15 min, centrifuge at 1500 g for 3 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 25 μL 6 mg/mL dinitrofluorobenzene in n-butyl acetate (prepare fresh each week, store at 4° in the dark), heat at 120° for 30 min, evaporate to dryness, reconstitute with 50 μL mobile phase, inject a 25-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm LiChrosorb RP18
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 80:20
Flow rate: 1
Injection volume: 25-50
Detector: UV 360

CHROMATOGRAM

Retention time: 5

Internal standard: glyburide (7)
Limit of detection: 2 ng/mL

KEY WORDS
derivatization; serum; ultrafiltrate; pharmacokinetics

REFERENCE
Arcelloni,C.; Fermo,I.; Calderara,A.; Pacchioni,M.; Pontiroli,A.E.; Paroni,R. Glibenclamide and tolbutamide in human serum: Rapid measurement of the free fraction, *J.Liq.Chromatogr.*, **1990**, *13*, 175–189.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 2 M HCl + 2 mL diethyl ether, vortex 30 s, centrifuge at 1500 g for 5 min, freeze in dry ice for 5 min. Decant ether layer and evaporate it to dryness under a stream of nitrogen at 35-40°. Reconstitute extract in 100 μL mobile phase, vortex 30 s, inject 25-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18
Mobile phase: MeCN:10 mM orthophosphoric acid 50:50
Flow rate: 1
Injection volume: 25-50
Detector: UV 230

CHROMATOGRAM
Retention time: 7.67
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: glyburide, gliclazide, chlorpropamide, glipizide, tolazamide
Noninterfering: trimethoprim, sulfamethoxazole

KEY WORDS
plasma

REFERENCE
Shenfield,G.M.; Boutagy,J.S.; Webb,C. A screening test for detecting sulfonylureas in plasma, *Ther.Drug Monit.*, **1990**, *12*, 393–397.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL water + 500 μL 100 mM HCl + 3 mL dichloromethane, mix for 15 s, centrifuge. Remove an aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 1000 × 8 10 μm radial pak C18 (Waters)
Mobile phase: MeOH:0.2% acetic acid 60:40 adjusted to pH 6.7 with 1 M NaOH (Wash with MeCN at 1 mL/min for 20 min at the end of each day.)
Flow rate: 0.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11
Internal standard: tolbutamide

OTHER SUBSTANCES
Extracted: chlorpropamide

KEY WORDS
plasma; tolbutamide is IS

REFERENCE
Bakare,M.T.; Mustapha,A.; Abdu-Aguye,I. An improved high-performance liquid chromatographic determination of chlorpropamide in human plasma, *Chromatographia*, **1994**, *39*, 107–109.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM
Retention time: 3.95
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil;

lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Plasma. 100 μL Plasma + 300 μL MeOH, mix, centrifuge at 3000 rpm for 5 min, filter (0.50 μm) the supernatant, inject an aliquot of the filtrate. Tissue. 500 μL Tissue homogenate + 2 mL MeOH, mix for 30 min, centrifuge at 4000 rpm for 20 min, filter (0.50 μm) the supernatant, inject an aliquot of the filtrate. Urine. Filter (0.45 μm) urine, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 TSKgel ODS-80TM (Tosoh)
Mobile phase: MeOH:isopropanol:0.05% phosphoric acid 35:6:60
Column temperature: 50
Detector: UV 227

CHROMATOGRAM

Limit of quantitation: 4 μg/mL

KEY WORDS

rat; pharmacokinetics; plasma; liver; small intestine; pharmacokinetics

REFERENCE

Yamao,T.; Nakagami,H.; Furuhama,K.; Onodera,T.; Kurosaki,Y.; Nakayama,T.; Kimura,T. Pharmacokinetics of tolbutamide following intravenous and oral administrations in rats with obstructive jaundice, *Biol.Pharm.Bull.*, **1994**, *17*, 691–695.

SAMPLE

Matrix: blood, urine

Sample preparation: Caution! Benzene is a carcinogen! 500 μL Plasma or urine + 1 mL 50 mM HCl + 3 mL benzene, shake gently for 15 min, centrifuge at 3250 g for 5 min. Remove organic layer and evaporate it to dryness under a stream of air. Dissolve residue in 50 μL mobile phase, vortex, inject 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm Spherisorb ODS C18
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM

Retention time: 8.2
Internal standard: tolbutamide

OTHER SUBSTANCES

Simultaneous: glipizide

KEY WORDS

plasma; tolbutamide is IS

REFERENCE

Emilsson,H. High-performance liquid chromatographic determination of glipizide in human plasma and urine, *J.Chromatogr.*, **1987**, *421*, 319–326.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or 200 μL urine + 100 μL 50 (plasma) or 250 (urine) μg/mL chlorpropamide in water + 100 μL 1 M HCl + 4 mL diethyl ether, shake for 10 min, centrifuge at 2000 g for 2-3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeCN:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm BST C8 (BST, Budapest)
Mobile phase: MeCN:isopropanol:0.1% orthophosphoric acid 17:17:66
Flow rate: 1.2
Injection volume: 5
Detector: UV 235

CHROMATOGRAM
Retention time: 10.5
Internal standard: chlorpropamide (8.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Csillag,K.; Vereczkey,L.; Gachalyi,B. Simple high-performance liquid chromatographic method for the determination of tolbutamide and its metabolites in human plasma and urine using photodiode-array detection, *J.Chromatogr.*, **1989**, *490*, 355–363.

SAMPLE
Matrix: microsomal incubation
Sample preparation: 1 mL Microsomal incubation + 100 μL 2 M HCl, cool rapidly on ice, extract with 8 mL hexane:chloroform:isobutyl alcohol 100:25:0.5, add 100 μL 10 μg/mL tolbutamide to the aqueous layer, extract with 3 mL diethyl ether. Combine (?) the organic layers and evaporate them to dryness at 60° under a stream of nitrogen, reconstitute with 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Mobile phase: MeCN:5 mM pH 4.3 acetate buffer 25:75
Flow rate: 1.5
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 15
Internal standard: chlorpropamide (8)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Rieutord,A.; Stupans,I.; Shenfield,G.M.; Gross,A.S. Gliclazide hydroxylation by rat liver microsomes, *Xenobiotica*, **1995**, *25*, 1345–1354.

SAMPLE
Matrix: microsomal incubations

Sample preparation: 200 μL Microsomal incubation + 25 μL 3 M HCl + 5 μL 1.78 μM chlorpropamide + 1 mL diethyl ether, vortex for 2 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL MeOH, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm C18 (Analytichem)
Mobile phase: MeOH:10 mM $(NH_4)H_2PO_4$ 55:45, pH 5.4
Flow rate: 0.7
Injection volume: 4
Detector: UV 240

CHROMATOGRAM
Retention time: 11
Internal standard: chlorpropamide (7)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Ho,J.W.; Moody,D.E. Determination of tolbutamide hydroxylation in rat liver microsomes by high-performance liquid chromatography: effect of psychoactive drugs on in vitro activity, *Life Sci.*, **1993**, *52*, 21–28.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 2 mL Microsomal incubation + 50 μL concentrated HCl + 100 μL 50 μg/mL chlorpropamide in MeOH + 4 mL chloroform, rotate for 30 min, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: Spherisorb ODS2 C18
Mobile phase: MeOH:0.05% orthophosphoric acid 50:50
Flow rate: 1
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 12.3
Internal standard: chlorpropamide (9.5)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Ashforth,E.I.L.; Carlile,D.J.; Chenery,R.; Houston,J.B. Prediction of *in vivo* disposition from *in vitro* systems: Clearance of phenytoin and tolbutamide using rat hepatic microsomal and hepatocyte data, *J.Pharmacol.Exp.Ther.*, **1995**, *274*, 761–766.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 200 μL 1 M orthophosphoric acid + 200 μL 2% chlorpropamide in water + 11 mL MTBE, extract for 40 min. Remove the organic layer and evaporate it to dryness under vacuum at 40°, reconstitute the residue in 25 μL MeCN:water 25:75, inject a 100 μL (sic) aliquot.

HPLC VARIABLES
Column: Novapack C18 radial compression
Mobile phase: MeCN:water 26:74
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 25.6
Internal standard: chlorpropamide (16.5)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Weaver,R.J.; Dickins,M.; Burke,M.D. Hydroxylation of the antimalarial drug 58C80 by CYP2C9 in human liver microsomes: Comparison with mephenytoin and tolbutamide hydroxylations, *Biochem.Pharmacol.*, **1995**, *49*, 997–1004.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic

acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.74 (A), 6.94 (B)

OTHER SUBSTANCES

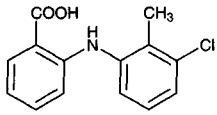
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline,

terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Tolfenamic acid



Molecular formula: $C_{14}H_{12}ClNO_2$
Molecular weight: 261.71
CAS Registry No.: 13710-19-5
Merck Index: 9650

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

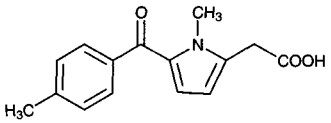
CHROMATOGRAM
Retention time: k' 3.31

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Tolmetin



Molecular formula: $C_{15}H_{15}NO_3$
Molecular weight: 257.29
CAS Registry No.: 26171-23-3, 64490-92-2 (sodium salt dihydrate), 35711-34-3 (sodium salt)
Merck Index: 9655
Lednicer No.: 2 234

SAMPLE

Matrix: blood
Sample preparation: 250 μL Plasma + 125 μL 1 M sulfuric acid + 5 mL 10 μg/mL diphenylacetic acid in dichloromethane, vortex for 10 s, centrifuge at 500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax ODS
Mobile phase: Gradient. MeOH:100 mM pH 5 acetate buffer 22:78 for 5 min, to 53:47 over 2 min, maintain at 53:47 for 8 min.
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 13.11
Internal standard: diphenylacetic acid (13.93)
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: acetaminophen, caffeine, carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone, quinidine, salicylic acid, theophylline
Noninterfering: fenoprofen, ibuprofen, indomethacin, naproxen, sulindac, valproic acid

KEY WORDS

plasma

REFERENCE

Shimek,J.L.; Rao,N.G.S.; Wahba Khalil,S.K. High performance liquid chromatographic analysis of tolmetin, indomethacin and sulindac in plasma, *J.Liq.Chromatogr.*, **1981**, *4*, 1987–2013.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 0.5 mL 1 M HCl + 10 mL dichloromethane, shake 10 min, centrifuge at 1000 g for 5 min. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute with 200 μL mobile phase, inject 10-30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 Partisil 10 ODS-3
Mobile phase: MeOH:water:phosphoric acid 700:300:1
Flow rate: 2.5
Injection volume: 10-30
Detector: UV 220

CHROMATOGRAM

Retention time: 3.2
Internal standard: tolmetin

OTHER SUBSTANCES
Simultaneous: ibuprofen, tolmetin is IS

KEY WORDS
plasma

REFERENCE
Lockwood,G.F.; Wagner,J.G. High-performance liquid chromatographic determination of ibuprofen and its major metabolites in biological fluids, *J.Chromatogr.*, **1982**, *232*, 335–343.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 90 μL 1 M HCl + 20 μL 1 mg/mL p-phenylphenol in MeCN, vortex for 10 s, add 6 mL benzene:tert-butyl alcohol 90:10 (Caution! Benzene is a carcinogen!), shake mechanically for 20 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL mobile phase, sonicate for a few s, inject an aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 30-40 μm Perisorb RP-18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:50 mM KH_2PO_4 48:52, pH adjusted to 4.7 with 8.5% phosphoric acid
Flow rate: 0.5 for 5 min then increase to 0.7 over 1 min, after another 4 min increase to 1.0 over 1 min
Injection volume: 20
Detector: UV 313

CHROMATOGRAM
Retention time: 6.5
Internal standard: p-phenylphenol (11)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: Med 5, Med 15, metabolites
Noninterfering: sulindac, naproxen, indomethacin, flufenamic acid, ibuprofen, aspirin

KEY WORDS
plasma; rat

REFERENCE
Mancinelli,A.; Bruno,G.; Cardace,G.; Morabito,E.; Marzo,A.; Arrigoni Martelli,E. High-performance liquid chromatographic evaluation of Med 15 and its metabolites Med 5 and tolmetin in rat plasma, *J.Chromatogr.*, **1991**, *553*, 81–86.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 100 mM pH 4 sodium acetate, extract twice with 5 mL diethyl ether. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL water, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:1 mM pH 3 phosphoric acid 32:68
Flow rate: 1
Injection volume: 80
Detector: UV 313

CHROMATOGRAM
Retention time: 11
Internal standard: tolmetin

OTHER SUBSTANCES
Extracted: ketorolac

KEY WORDS
plasma; pharmacokinetics; tolmetin is IS

REFERENCE
Flores-Murrieta,F.J.; Granados-Soto,V.; Castañeda-Hernández,G.; Herrera,J.E.; Hong,E. Comparative bioavailability of two oral formulations of ketorolac tromethamine: Dolac and Exodol, *Biopharm.Drug Dispos.*, **1994**, *15*, 129–136.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL pH 1.8 phosphate buffer, extract with 1-butanol/MTBE. Remove the organic layer and add it to 500 μL pH 6.1 ammonium acetate buffer, mix, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil C18
Mobile phase: MeCN:250 mM pH 5.0 ammonium acetate buffer 20:80
Flow rate: 1.8
Detector: UV 258

CHROMATOGRAM
Internal standard: tolmetin

OTHER SUBSTANCES
Extracted: ketoprofen (UV 350)

KEY WORDS
plasma; tolmetin is IS

REFERENCE
Shah,A.K.; Wei,G.; Lanman,R.C.; Bhargava,V.O.; Weir,S.J. Percutaneous absorption of ketoprofen from different anatomical sites in man, *Pharm.Res.*, **1996**, *13*, 168–172.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 322

OTHER SUBSTANCES
Also analyzed: carbamazepine, fenbufen, indomethacin, ketoprofen, α.-naphthoquinone, naproxen

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:10 mM sodium carbonate 18:82. B was MeCN:50 mM sodium carbonate 33:67. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: aspirin, ibuprofen, naproxen, fenbufen, indomethacin, carprofen, diflunisal

REFERENCE

Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, predni-

solone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.45 (A), 6.29 (B)

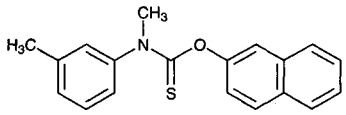
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Tolnaftate



Molecular formula: $C_{19}H_{17}NOS$
Molecular weight: 307.42
CAS Registry No.: 2398-96-1
Merck Index: 9656
Lednicer No.: 2 211

SAMPLE
Matrix: blood, tissue
Sample preparation: Skin. Homogenize 100-200 mg skin with 3 mL 1.15% KCl, add 3 mL naphthyl phenylacetate in dichloromethane (?), add 3 mL dichloromethane, agitate for 10 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 10 μL aliquot. Whole blood. 200 μL Whole blood + 200 μL water, mix, add 3 mL naphthyl phenylacetate in dichloromethane (?), add 3 mL dichloromethane, agitate for 10 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:water 80:20

KEY WORDS
whole blood; skin; mouse; pharmacokinetics

REFERENCE
Szeman,J.; Ueda,H.; Szejtli,J.; Fenyvesi,E.; Watanabe,Y.; Machida,Y.; Nagai,T. Enhanced percutaneous absorption of homogenized tolnaftate/β-cyclodextrin polymer ground mixture, *Drug Des.Deliv.*, **1987**, *1*, 325–332.

SAMPLE
Matrix: formulations
Sample preparation: Solutions. Measure out amount containing 10 mg tolnaftate, dilute to 100 mL with MeCN:water 80:20, dilute 10 fold with MeCN:water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate. Aerosol liquids. Discharge aerosol into a container, warm at 25° until propellant dissipates, dilute a 1 mL aliquot to 100 mL with MeCN: water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate. Powders. Weigh out amount containing 5 mg tolnaftate, add 50 mL MeCN:water 80:20, shake mechanically for 15 min, make up to 100 mL with MeCN:water 80:20, let stand for 15 min, dilute an aliquot 5 fold with MeCN:water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate. Aerosol powders. Discharge aerosol into a container, heat at 25° under a current of air to remove propellant, weigh out amount containing 5 mg tolnaftate, add 50 mL MeCN:water 80:20, shake mechanically for 15 min, make up to 100 mL with MeCN: water 80:20, let stand for 15 min, dilute an aliquot 5 fold with MeCN:water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate. Creams, gels. Weigh out amount containing 5 mg tolnaftate, add 50 mL MeCN:water 80:20, shake mechanically for 15 min, make up to 100 mL with MeCN:water 80:20, let stand for 15 min, dilute an aliquot 5 fold with MeCN:water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 80:20 containing 500 μL/L phosphoric acid, pH 2.4
Flow rate: 1
Injection volume: 20
Detector: UV 257

CHROMATOGRAM
Retention time: 5

KEY WORDS
liquids; powders; aerosols; solutions; cream; gels

REFERENCE
Thompson,R.D.; Carlson,M. Liquid chromatographic determination of tolnaftate in commercial products, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 603–607.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μg/mL solution in MeOH, inject an aliquot.

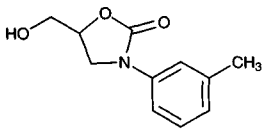
HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select-B (A) or 125 × 2 4 μm Superspher 100 RP-18 (B)
Mobile phase: MeCN:pH 2 trifluoroacetic acid 70:30 (A) or Gradient. C was MeCN:water 90:10. D was MeOH:pH 2 trifluoroacetic acid buffer 15:85. C:D from 30:70 to 80:20 over 8 min (B)
Flow rate: 1 (A) or 0.4 (B)
Detector: UV 254 (A), UV 245 (B)

CHROMATOGRAM
Retention time: 9.75 (A), 9.6 (B)

OTHER SUBSTANCES
Simultaneous: cloxyquin, chlorphenesin, naftifine, sulbentine, degradation products

REFERENCE
Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

Toloxatone



Molecular formula: $C_{11}H_{13}NO_3$
Molecular weight: 207.23
CAS Registry No.: 29218-27-7
Merck Index: 9659

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 238

CHROMATOGRAM

Retention time: 3.35
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF
Sample preparation: 50 μL Plasma or CSF + 50 μL 2 μg/mL trazodone, vortex briefly, add 20 μL 4 M NaOH, vortex briefly, add 750 μL diethyl ether, vortex for 1 min, centrifuge at 2600 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 25 × 4 Hibar LiChroCART C8 (Merck)
Column: 250 × 4 5 μm LiChrospher 100 CH-8 II C8
Mobile phase: MeCN:10 mM pH 3.0 phosphate buffer 60:40 containing 20 mM tetramethylammonium chloride
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 4.9
Internal standard: trazodone (6.0)
Limit of detection: 70 ng/mL

KEY WORDS

plasma; rabbit; pharmacokinetics

REFERENCE

Vistelle,R.; Lamiable,D.; Zinzou,M. Simple high-performance liquid chromatographic method for the measurement of toloxatone in rabbit cerebrospinal fluid and plasma, *J.Chromatogr.*, **1989**, *490*, 387–394.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM

Retention time: 14.107

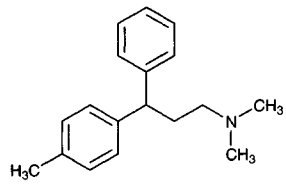
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tolpropamine



Molecular formula: $C_{18}H_{23}N$
Molecular weight: 253.39
CAS Registry No.: 5632-44-0
Merck Index: 9662

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

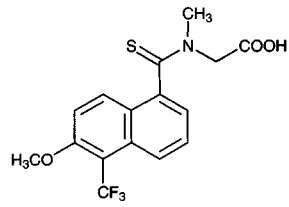
Retention time: 3.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Tolrestat



Molecular formula: $C_{16}H_{14}F_3NO_3S$
Molecular weight: 357.35
CAS Registry No.: 82964-04-3
Merck Index: 9663
Lednicer No.: 4 56

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. 1 mL Serum + 4 mL 1 M HCl + 10 mL isopropyl ether (Caution! isopropyl ether readily forms explosive peroxides!), agitate mechanically for 15 min, centrifuge at 1500 rpm for 10 min. Remove 8.5 mL of the upper organic phase and add it to 1 mL 100 mM pH 11.0 glycine buffer, agitate mechanically for 15 min, centrifuge at 1500 rpm for 10 min. Remove 800 μL of the aqueous phase and add it to 20 μL 2.5 M phosphoric acid, inject a 50-150 μL aliquot. (For increased sensitivity use 2 mL serum and 500 μL glycine buffer.) Tissue. Homogenize (all-glass, Kontes) 50-100 mg lens or nerve tissue with 3 mL isotonic saline. 2.5 mL Homogenate + 4 mL 100 mM phosphoric acid + 10 mL isopropyl ether (Caution! isopropyl ether readily forms explosive peroxides!), agitate mechanically for 30 min, centrifuge at 1500 rpm for 15 min. Remove 9.0 mL of the upper organic phase and add it to 1 mL 100 mM pH 11.0 glycine buffer, agitate mechanically for 15 min, centrifuge at 1500 rpm for 10 min. Remove 800 μL of the aqueous phase and add it to 20 μL 2.5 M phosphoric acid, inject a 50-150 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:100 mM pH 6.0 phosphate buffer 30:70
Column temperature: 50
Flow rate: 1.1
Injection volume: 50-150
Detector: UV 226

CHROMATOGRAM
Retention time: 6.4
Limit of detection: 25 ng/mL (2 mL serum), 200 ng/mL (1 mL serum), 50 ng/g (tissue)

OTHER SUBSTANCES
Extracted: rotamer
Noninterfering: acetaminophen, diazepam, glyburide, hydrochlorothiazide, indomethacin, niacin, phenobarbital, phenytoin, propoxyphene, salicylic acid, tolbutamide
Interfering: dicoumarol, phenylbutazone

KEY WORDS
rat; dog; human; serum; sciatic nerve; lens

REFERENCE
Hicks,D.R.; Kraml,M. Determination of tolrestat, a novel aldose reductase inhibitor, in serum and tissues, *Ther.Drug Monit.*, **1984**, *6*, 328–333.

SAMPLE
Matrix: bulk

Sample preparation: Prepare a 100 μg/mL solution in MeCN:buffer 35:65, inject a 5 μL aliquot. (Buffer was 10 mM phosphoric acid adjusted to pH 7.0 with 10 mM ammonium hydroxide.)

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve octadecylsilane (Waters)
Mobile phase: MeCN:THF:buffer:40% tetrabutylammonium hydroxide in water 20.5:18.5:61.5:0.3 (Buffer was 50 mM $(NH_4)H_2PO_4$ adjusted to pH 3.5 with 50 mM phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 229

CHROMATOGRAM
Retention time: 4.6
Limit of quantitation: 0.05% (of tolrestat)

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
rugged

REFERENCE
Beaulieu,N.; Lacroix,P.M.; Sears,R.W.; Lovering,E.G. Liquid chromatographic determination of tolrestat and related compounds in raw materials, *J.AOAC Int.*, **1995**, *78*, 647–650.

SAMPLE
Matrix: solutions
Sample preparation: Inject buffer solution directly.

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM pH 6.5 phosphate buffer 30:70 containing 0.8 g/L tetraethylammonium chloride (Flush column with MeOH at the end of each day.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7 (rotamer A), 3.9 (rotamer B)

OTHER SUBSTANCES
Simultaneous: rotamers

KEY WORDS
buffer

REFERENCE
Lee,H.-K.; Querijero,G. Kinetics and mechanisms of thioamide rotational isomerism: *N*-thionaphthoyl-*N*-methyl glycine derivative, *J.Pharm.Sci.*, **1985**, *74*, 273–276.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

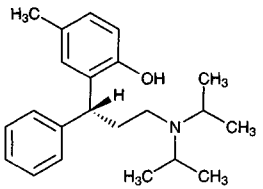
HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM KH_2PO_4:1 M tetrabutylammonium hydroxide 35:65:0.5
Flow rate: 1.5
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 8 (rotamer A), 6 (rotamer B)

OTHER SUBSTANCES
Simultaneous: degradation products, rotamers

REFERENCE
Lee,Y.J.; Lee,H.-K. Degradation kinetics of tolrestat, *J.Pharm.Sci.*, **1990**, *79*, 628–633.

Tolterodine



Molecular formula: $C_{22}H_{31}NO$
Molecular weight: 325.49
CAS Registry No.: 124937-51-5

SAMPLE
Matrix: blood
Sample preparation: Mix plasma or serum with two volumes of acetone, centrifuge. Remove the supernatant, wash the pellets with two portions of acetone:20 mM pH 4.5 ammonium acetate 50:50, evaporate the combined supernatant and pellet extract to dryness, dissolve the residue in MeOH:20 mM pH 4.5 ammonium acetate 10:90. Inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.5 Supelco PKB 100
Column: 150 × 4.5 Supelco PKB 100
Mobile phase: Gradient. A was MeOH. B was 20 mM pH 4.5 ammonium acetate. A:B from 10:90 to 20:80 in 5 min, from 20:80 to 45:55 in 30 min, from 45:55 to 100:0 in 5 min, maintain at 100:0 for 10 min
Flow rate: 1
Injection volume: 100
Detector: UV 280

CHROMATOGRAM
Retention time: 29

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
dog; mouse; serum; plasma

REFERENCE
Andersson,S.H.G.; Lindgren,A.; Postlind,H. Biotransformation of tolterodine, a new muscarinic receptor antagonist, in mice, rats, and dogs, *Drug Metab.Dispos.*, **1998**, *26*, 528–535.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Mix 250 μL or 1 mL microsomal incubation with an equal volume of acetone, centrifuge at 3200 rpm, evaporate the acetone in the supernatant under a stream of nitrogen. Inject a 100-200 μL aliquot of the remaining supernatant.

HPLC VARIABLES
Guard column: 20 mm Supelco PKB 100
Column: 150 × 4.5 Supelco PKB 100

Mobile phase: Gradient. A was MeOH. B was 20 mM pH 4.5 ammonium acetate. A:B from 10:90 to 20:80 over 5 min, to 45:55 over 30 min, to 100:0 over 5 min, maintain at 100:0 for 10 min
Flow rate: 1
Injection volume: 100-200
Detector: UV 280; Radioactivity, Beckman 171 radioisotope detector

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
pharmacokinetics; liver

REFERENCE
Postlind,H.; Danielson,Å.; Lindgren,A.; Andersson,H.,G. Tolterodine, a new muscarinic receptor antagonist, is metabolized by cytochromes P450 2D6 and 3A in human liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 289–293.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add an equal volume of acetone to the microsomal incubation, centrifuge at 3200 rpm, evaporate acetone under a stream of nitrogen. Inject a 100-200 μL aliquot of the remaining supernatant.

HPLC VARIABLES
Guard column: 20 mm long Supelco PKB 100
Column: 150 × 4.5 Supelco PKB 100
Mobile phase: Gradient. A. MeOH. B. 20 mM ammonium acetate. A:B from 10:90 to 20:80 over 5 min, to 45:55 over 30 min, to 100:0 over 5 min, maintain at 100:0 for 10 min.
Flow rate: 1
Injection volume: 100-200
Detector: UV 280

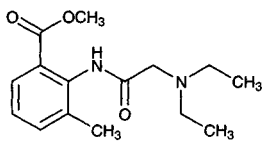
CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
liver

REFERENCE
Postlind,H.; Danielson,Å.; Lindgren,A.; Andersson,H.,G. Tolterodine, a new muscarinic receptor antagonist, is metabolized by cytochromes P450 2D6 and 3A in human liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 289–293.

Tolycaine



Molecular formula: $C_{15}H_{22}N_2O_3$
Molecular weight: 278.35
CAS Registry No.: 3686-58-6, 7210-92-6 (HCl)
Merck Index: 9679
Lednicer No.: 1 17

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

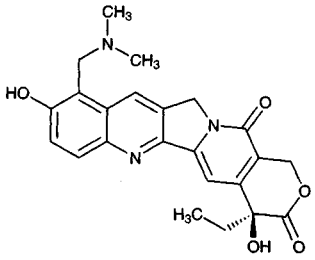
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Topotecan



Molecular formula: $C_{23}H_{23}N_3O_5$
Molecular weight: 421.45
CAS Registry No.: 123948-87-8, 119413-54-6 (HCl)
Merck Index: 9687

SAMPLE
Matrix: formulations
Sample preparation: Dilute topotecan hydrochloride injection with 5% dextrose or 0.9% NaCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5C18-AR (Nacalai Tesque, Japan)
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 15:85:0.1. B was MeCN:water:trifluoroacetic acid 40:60:0.1. A:B from 100:0 to 0:100 in 20 min, from 0:100 to 100:0 in 1 min
Flow rate: 1
Injection volume: 40
Detector: UV 228

CHROMATOGRAM
Retention time: 10.0

KEY WORDS
injections

REFERENCE
Craig,S.B.; Bhatt,U.H.; Patel,K. Stability and compatibility of topotecan hydrochloride for injection with common infusion solutions and containers, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 199–205.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute 4-mg topotecan hydrochloride injection with water to a nominal concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5C18-AR (Nacalai Tesque, Kyoto)
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 15:85:0.1. B was MeCN:water:trifluoroacetic acid 40:60:0.1. A:B 100:0 for 16 min, to 0:100 over 24 min, to 100:0 over 2 min, maintain at 100:0 for 18 min
Flow rate: 1
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 10.7-12.0

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Patel,K.; Craig,S.B.; McBride,M.G.; Palepu,N.R. Microbial inhibitory properties and stability of topotecan hydrochloride injection, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1584–1587.